UNCLASSIFIED

2

REPORT DOCUMENTATION PAGE

AD-A211 220

1b. RESTRICTIVE MARKINGS

3. DISTRIBUTION / AVAILABILITY OF REPORT
Approved for public release; distribution unlimited.

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
SR89-20

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
Armed Forces Radiobiology Research Institute

6b. OFFICE SYMBOL (If applicable)
AFRRI

7a. NAME OF MONITORING ORGANIZATION

6c. ADDRESS (City, State, and ZIP Code)
Bethesda, MD 20814-5145

7b. ADDRESS (City, State, and ZIP Code)

8a. NAME OF FUNDING / SPONSORING ORGANIZATION
Defense Nuclear Agency

8b. OFFICE SYMBOL (If applicable)
DNA

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State, and ZIP Code)
Washington, DC 20305

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
NWED QAXM			B4163

11. TITLE (Include Security Classification)
(see reprint)

12. PERSONAL AUTHOR(S)
Mickley, G. A., and Ferguson, J. L.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Reprint	FROM ____ TO ____	1989	7

16. SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

DTIC

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION

22a. NAME OF RESPONSIBLE INDIVIDUAL
Gloria Ruggiero

22b. TELEPHONE (Include Area Code)
(202) 295-2017

22c. OFFICE SYMBOL
ISDP

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

Exp Brain Res (1989) 75: 28–34

Experimental Brain Research

Enhanced acoustic startle responding in rats with radiation-induced hippocampal granule cell hypoplasia

G. A. Mickley and J. L. Ferguson

Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145, USA

Summary. Irradiation of the neonatal rat hippocampus reduces the proliferation of granule cells in the dentate gyrus and results in locomotor hyperactivity, behavioral perseveration and deficits on some learned tasks. In order to address the role of changes in stimulus salience and behavioral inhibition in animals with this type of brain damage, irradiated and normal rats were compared in their startle reactions to an acoustic stimulus. A portion of the brain of 10 rats was exposed to a fractionated total dose of 13 Gy during the first 16 days post partum. This procedure produced selective hypoplasia (91% reduction) of the granule cells in the hippocampal dentate gyrus. Other rats (N = 10) were sham irradiated. Sudden tones were presented to each adult rat at a rate of 1 every 30 s (spaced trials) during an initial 10-min session and 1 every 15 s (massed trials) during a subsequent session. Irradiated rats startled with a consistently higher amplitude than controls and were more likely to exhibit startle responses. These animals with hippocampal damage also failed to habituate to the startle stimulus and, under certain circumstances, showed potentiated startle responses after many tone presentations.

Key words: Startle – Hippocampus – Dentate gyrus – Granule cells – Radiation

Introduction

The startle response consists of a characteristic sequence of rapid muscular contractions beginning at the mouth and sequentially involving the neck, forelegs and finally the whole body (Landis and Hunt 1939). Analyses of movements associated with startle are being used increasingly in the study of human behavior (Wilkins et al. 1986) and the behavior of other animals (Eaton 1984). Although the acoustic startle response is a relatively simple behavior, its sensitivity to a variety of experimental treatments has made it an important tool in pharmacology and toxicology (for review see Eaton 1984). In particular, brain mechanisms of sensation, learning (habituation, sensitization), memory and movement are being elucidated through measures of startle (Davis 1984).

The neurons that comprise the primary acoustic startle circuit reside entirely within the brain stem (Davis 1984). However, the nature of the extrinsic neural systems that modulate acoustic startle is not so well understood. Since the hippocampus has long been known to play a role in response inhibition (Douglas 1967; Kimble 1968; Altman et al. 1973) it is likely that this structure also influences startle responding. In support of hippocampally mediated behavioral inhibition other investigators have reported excitatory behaviors after hippocampal lesions. For example, rats with hippocampal lesions exhibit locomotor hyperactivity (Teitelbaum and Milner 1963; Means et al. 1971), response perseveration (Isaacson 1974), facilitated acquisition of active avoidance (Isaacson et al. 1961) and impaired performance on passive avoidance tasks (Blanchard and Fial 1968; Isaacson and Wickelgren 1962). More-selective hippocampal lesions of CA3 have also been reported to produce hyper-reactivity to sensory stimulation (Handelmann and Olton 1981). Still, the function of the hippocampus in startle responding is controversial. Some authors (Groves et al. 1974; Leaton 1981) have reported that lesions of the hippocampus do not consistently alter startle, while others (Coover and Levine 1972) have found increased acoustic startle after surgically induced hippocampal damage.

Offprint requests to: G. A. Mickley (address see above)

Bayer et al. (1973) have identified a number of similarities between the behavioral deficits observed in rats with selective hippocampal granule cell lesions and the behavioral dysfunctions found after hippocampectomy. This is not totally surprising since the perforant path (from the entorhinal cortex to the granule cells of the dentate gyrus) provides one of the major inputs to the hippocampus (O'Keefe and Nadel 1978). Lesions, specific to the granule cells, effectively reduce the targets of these entorhinal cortex neurons, significantly limit hippocampal afferents and disrupt a variety of hippocampal functions (Brunner and Altman 1974; Altman 1986; Wallace and Altman 1970a, b).

In the present experiment we produced granule cell hypoplasia in the fascia dentata by partial-head X-irradiation of neonatal rats (Bayer and Peters 1977). In order to address the role of stimulus salience and behavioral inhibition in animals with these selective hippocampal lesions, irradiated and normal adult rats were compared in their startle reactions to an acoustic stimulus.

Material and methods

Time pregnant female Crl : CD(SD)BR rats were purchased from Charles River Laboratories, Kingston, NY, for these experiments. Pregnant rats were quarantined on arrival and screened for evidence of disease. Upon release from quarantine, they were maintained in a facility accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). Subjects were housed in Micro-isolator cages on hardwood chip contact bedding and provided with commercial rodent chow and acidified water ad libitum (Weisbroth 1979). Animal holding rooms were maintained at 19–21° C with 50% ± 10% relative humidity using at least 10 air changes per hour of 100% conditioned fresh air. The rats were on twelve hour light/dark full-spectrum lighting cycle with no twilight. On the day of birth, litters were culled to include only males. Neonatal subjects were then randomly assigned to either the X-irradiated or the sham-irradiated (control) conditions. The sham-irradiated control rats were restrained under lead shielding in the same manner as the irradiated animals (see below) but were not exposed to X rays. Following weaning (at 24–30 days) rats were individually housed.

Portions of the brains of the experimental rats were exposed to 2.0 Gy X rays on postnatal days 1 and 2, and to 1.5 Gy on days 5, 7, 9, 12, 14, and 16. X irradiation was delivered at a rate of 0.49 Gy/minute at a depth of 2 mm in tissue. The radiation source was a Phillips Industrial 300-kvp X ray machine (Phillips Inc. Mahwah, N.J.) configured with 1.5 mm of copper filtration. The half-value layer was 2.5 mm copper. Doses were determined by using Exradin 0.05 cc tissue equivalent ion chambers with calibration traceable to the National Bureau of Standards. Following a procedure similar to that developed by Bayer and Peters (1977) collimated X rays were delivered dorsally (in the coronal plane) to those areas of the brain previously determined to contain the hippocampus. The X-ray exposure occurred through a slot in a whole-body lead shield. The slot width ranged from 7–12 mm (measured in the anterior/posterior plane) in order to accomodate the growth of the head/brain over the course of the radiation treatments. The entire anterior-posterior extent of the hippocampus was irradiated as were brain areas dorsal to and ventral to this structure (Paxinos and Watson 1982). Brain areas anterior and posterior to the slot were shielded.

Despite the fact that much of the neonatal rat brain was exposed to X rays, only the precursors of the granule cells in the dentate gyrus were permanently altered by this procedure (Hicks 1958; Bayer and Peters 1977). Most of the rat brain develops prenatally. At the time of our radiation exposures the brain contains only 3 populations of dividing (and therefore radiosensitive) neuronal precursors: the granule cells of the hippocampus, cerebellum and olfactory bulbs (Bayer et al. 1973; Bayer and Altman 1975). Through our shielding we protected two of these neuronal precursor populations (in the cerebellum and olfactory bulbs). Our X rays hit only the mitotic (radiosensitive) granule cells of the dentate gyrus and the mature neurons (that are radioresistant, see Cassaret 1980; Hicks and D'Amato 1966) in other brain structures residing in the same coronal plane as the hippocampus. This procedure produces selective hypoplasia of granule cells in the dentate gyrus while sparing other brain structures. The technique has been validated through a variety of neuroanatomical methods (Hicks 1958; Bayer and Altman 1975; Zimmer et al. 1985) including the ones reported here (see below).

Acoustic startle was measured on a Columbus Instrument's (Columbus, OH) Responder IV within a sound-attenuating chamber (Model E10-20, Coulbourn Instruments, Lehigh Valley, PA). The startle apparatus consisted of a (15 × 30 cm) metal plate mounted on load cells allowing a measurement ("amplitude" of response) directly proportional to a sudden force applied to the plate. The rat to be tested was placed in a transparent plastic box (10 × 12 × 22 cm) with a perforated lid. The rat could sit in the box without touching the top or sides of the container. Subjects did not locomote within the startle chamber. The box was set on the sensing plate 2 cm beneath a speaker from which a 10 ms acoustic stimulus (90 dB, SPL; 10 kHz) was presented.

An equal number of experimental (N = 10) and control (N = 10) rats were selected from the same litters and allowed to mature (mean age = 210 days; SD = 39). Before behavioral testing, subjects were matched for weight (irradiated rat mean = 656 g, SD = 36; control rat mean = 684 g, SD = 28). Each rat was placed in the plastic chamber which was then set immediately on the sensing plate of the startle apparatus. During the first session (spaced trials) the sound pulse was presented every 30 s for 10 min. During the second session, 4 days later (massed trials), the stimulus was presented every 15 s for the 10 min. Relative amplitude measurements were recorded after each trial. The sensitivity on the startle apparatus was set at 0.5 (5% of full scale) with a sampling window of 50 ms following stimulus onset. Movements were not recorded as a "startle" if they failed to be detected within these measurement parameters. Output linearity and stability were confirmed by recording output amplitudes after dropping small weights (5 to 35 g) from 2 cm to the sensor plate, or by dropping a 40 g mass 0.6 cm to the plate when it was loaded with 550 to 1250 g of dead weight. The mean startle amplitude recorded for the 20 subjects was 548; this was equivalent to dropping a 17 g weight 2 cm to the non-loaded sensor plate.

Using irradiation procedures similar to ours, Bayer and Peters (1977) have previously determined that X irradiation destroys approximately 85% of the granular cells in the dentate gyrus of the hippocampus while sparing adjacent structures (Bayer et al. 1973). After behavioral testing our rats were anesthetized and perfused with heparinized saline followed by 10% buffered formalin. Brains were embedded in paraffin, serially-sectioned (6 μ) (in either the coronal or sagittal plane) and then stained with cresyl violet and luxol fast blue (LaBossière 1976). All brains were viewed to confirm radiation-induced changes in the hippocampus. In addition, irradiated and sham-irradiated brains were selected at

Table 1. Histological data derived from analysis of sagittal sections of hippocampus

	Irradiated (N = 9)	Sham Irradiated (N = 6)	% of control
Number of dentate granule cells	177.2 (27.9)[a]	1966.2 (122)[b]	9%
Dentate area [sq mm]	0.4 (0.06)[a]	2.1 (0.02)	19%
Density of dentate granule cells [/sq mm]	472.5 (43.8)[a]	971.3 (56.2)	48%
Thickness[c] of dentate granule cell layer	3.5 (0.2)[a]	8.1 (0.5)	43%
Thickness[c] of CA1 pyramidal cell layer	2.8 (0.2)	2.8 (0.2)	100%

[a] Difference from sham-irradiated $P < 0.001$
[b] Numbers are means with SEM in parentheses
[c] Number of cells

random for a further cell-counting analysis. One or two sections from each brain were selected for this review. Sagittal sections (used to count granule cells in the olfactory bulb, hippocampus and cerebellum) were 1.9 mm lateral from the midline. Coronal sections, used to count hippocampal and cerebellar granule cells, were 3.3 mm and 11.8 mm posterior to bregma, respectively (Paxinos and Watson 1982). We counted total granular cells in the dentate gyrus. Using an imaging system (Bioquant System IV, R & M Biometrics, Inc., Nashville, TN) we also derived the area of the dentate gyrus, computed the cellular density of the structure and the thickness of the granule cell layer. In order to confirm that the shielding of other brain areas was sufficient, we also counted granule cells in a 0.004 mm^2 area in the cerebellum and olfactory bulb and measured the area of the entire cerebellum. Further, we evaluated the sparing of other more-mature, and therefore less-radiosensitive, hippocampal structures by counting the thickness of the CA1 pyramidal cell layer that was dorsal to the dentate and directly in the path of the X radiation. Unless otherwise stated, statistical analyses of histological findings used data from the sagittal sections (see Table 1).

Results

Our histological and behavioral data suggest that hippocampal granule cell hypoplasia enhances startle amplitude and frequency and limits startle habituation.

Exposure of the neonatal rat hippocampus to ionizing radiation produced a significant [$t(6) = 14.3$, $P < 0.001$] depletion of dentate granule cells (Table 1, Fig. 1). This damage was fully quantified only in the brains randomly selected for cell counting but was easily observed in all irradiated brains. Similarly, both the areas and the granule cell densities of the irradiated dentate gyri were significantly reduced compared to those of the control rats [$t(13) = 11.0$, $P < 0.001$ and $t(13) = 7.1$, $P < 0.001$, respectively]. The specificity of this damage is illustrated by the sparing of the post-mitotic pyramidal CA1 neurons that were directly in the path of the X rays. Irradiation produced no change in the thickness of the CA1 pyramidal cell layer, yet the thickness of the dentate granule cell layer was significantly reduced [$t(13) = 9.4$, $P < 0.001$]. The granule cell populations (i.e., number of cells/unit area) of the olfactory bulb and the cerebellum were not significantly altered by the irradiation although there was a slight trend toward more cells in these structures in irradiated rats. Further, exposure to ionizing radiation did not change the total area of the cerebellum when measured in coronal section. These data suggest that the shielding of the olfactory bulb and cerebellum during the irradiation treatment was effective.

Although there were different cell counts associated with the coronal and sagittal planes of section, the histological data derived from sections in either of these planes generally suggested identical conclusions. However, when we used sagittal sections to determine the total area of the cerebellum, this analysis revealed a radiation-induced reduction in overall cerebellar size [$t(13) = 2.7$, $P < 0.1$] whereas the review of the coronal sections did not indicate this difference. This fact, in itself, is not remarkable since others (Bayer and Altman 1975), have reported different results from measures of hippocampal anatomy depending on the plane of the brain section analyzed. The cerebellum was shielded during irradiation, the density of the granule cells present was normal and the organization of cells within this structure did not resemble that reported to occur when this brain area is exposed to ionizing radiation (Brunner and Altman 1974; Altman 1986). Further, a reduction in cerebellar height (in the coronal plane) has been observed in [illegible] with radiation-induced cerebellar damage (Altman et al. 1968) but was not present in our subjects. It may be that reduced cerebellar size in the sagittal plane, but not the coronal plane, is due to overall cranial shortening since even partial head irradiation can reduce bone growth (both within and outside the head) (Mosier and Jansons 1970).

Although the origin of the singular cerebellar change reported here is currently unclear, cerebellar damage probably played a negligible role in the production of the behavioral results reported below. There is a dissimilarity between the behaviors charac-

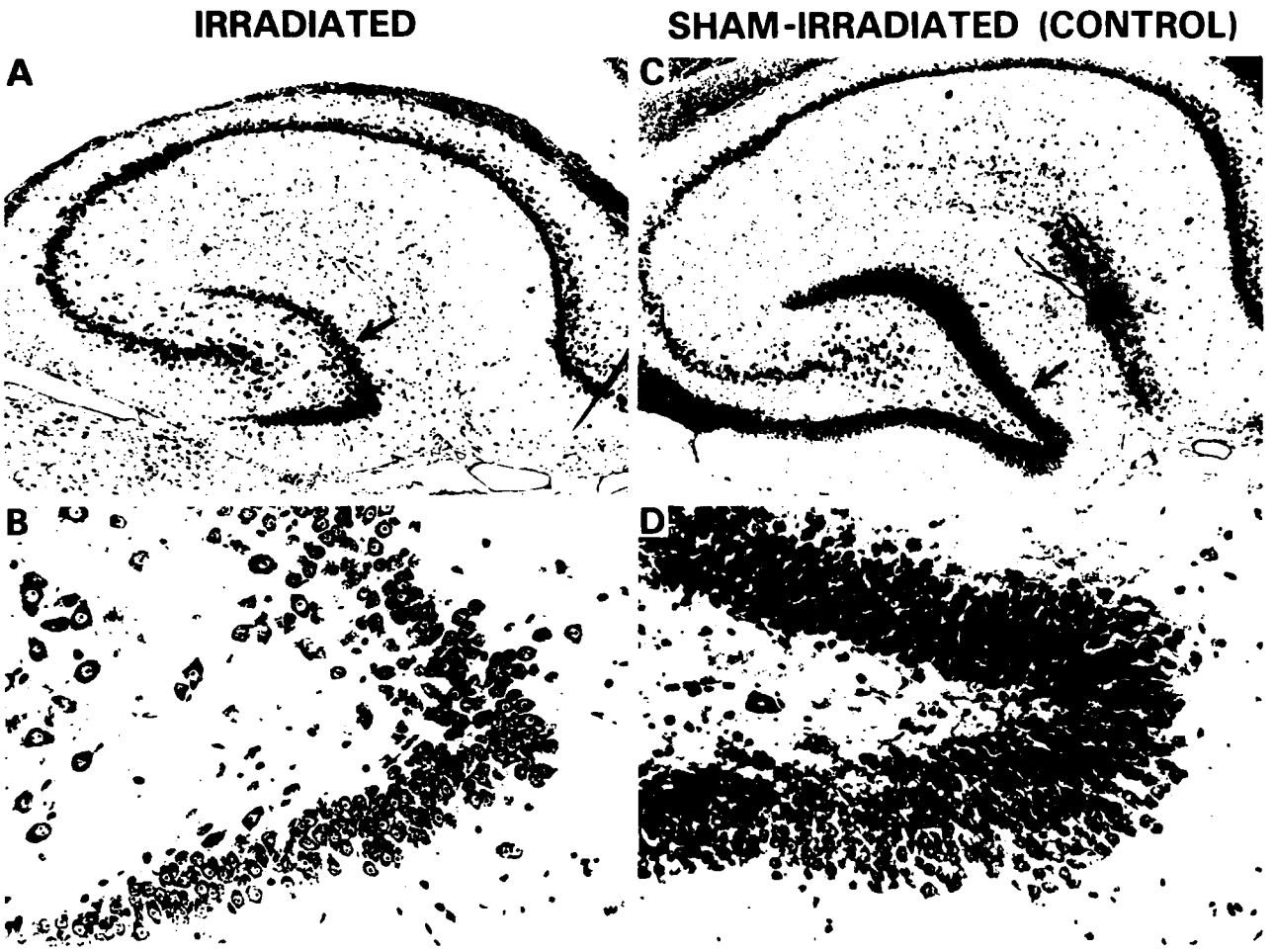

Fig. 1. Sagittal sections of hippocampus from either an irradiated (**A**, **B**) or sham-irradiated control rat (**C**, **D**). Neonatal exposure to X rays (using a procedure similar to that of Bayer and Peters 1977) produced a significant reduction in size, cell number, and thickness of the granule cell layer of the dentate gyrus (arrow) while sparing other adjacent brain structures. Enlargements (**B**, **D**) show the apex of the dentate gyrus from the brain section directly above

teristic of cerebellar and hippocampal damage. For example, locomotor hypoactivity, observed in rats with radiation-induced cerebellar damage (Wallace and Altman 1970 a, b; Brunner and Altman 1974) was not seen in rats with the same brain lesions as those reported here (Mickley et al. 1989).

Acoustic startle was markedly enhanced in subjects with radiation-induced hippocampal damage (see Fig. 2). This increase in amplitude occurred within spaced trials (tone presented each 30 s during the first session) [Mann-Whitney U(150, 158) = 14981, $P < 0.001$] and within massed trials (tone presented each 15 s during the second session) [U(123, 198) = 16031, $P < 0.001$].

Rats in the experimental and control groups were matched for body weight in order to attenuate any bias that this factor might have on the startle measure. However, in order to confirm our results of enhanced startle in subjects with hippocampal damage we performed an analysis of covariance which adjusted for the weight of the subjects. Within this analysis, it was clear that the mean startle responses exhibited by irradiated rats were significantly higher during both the first [F(1,17) = 7.817, $P = 0.012$] and second [F(1,17) = 7.452, $P = 0.014$] test sessions. Further, when we adjusted the data for the effect produced by the irradiation treatment it was apparent that heavier animals tended to have consistently higher startle responses: [session 1: F(1,17) = 8.356, $P = 0.01$; session 2: F(1,17) = 8.699, $P = 0.009$]. Although animal weight can influence acoustic startle measurements, our observation that startle is enhanced in rats with hippocampal damage was not confounded by this variable.

The apparent decline in acoustic startle (Fig. 2) between the first and second halves of session 1

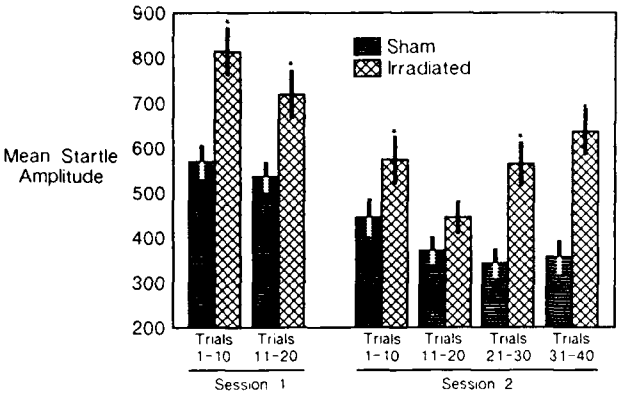

Fig. 2. Mean amplitude of acoustic startle responses exhibited by irradiated rats with hippocampal granule cell hypoplasia and control rats. Results from ten trial blocks are shown for each of two test sessions. In session 1, tone presentations were spaced (1/30 s) while in sessison 2 they were more frequent (massed at a rate of 1/15 s). Irradiated rats produced startle responses of greater amplitudes than those of controls. Through the course of the second session, habituation was observed in sham-irradiated rats but not in rats with hippocampal damage. Dispersion indicators are the standard errors of the means. (* represents a statistically significant difference between the experimental and control groups at a particular time period, $P < 0.05$, Mann-Whitney U)

(spaced trials) was not statistically significant for either experimental or control animals (Mann-Whitney U). The responses in the first and second halves of session 2 (massed trials) revealed a statistically significant decline in the startles exhibited by the sham-irradiated rats [$U(67,56) = 2217$, $P = 0.04$]. However, for the irradiated rats with hippocampal damage, not only did startle amplitude fail to habituate during the second session but there was a trend toward potentiation of this response.

Because a rapid habituation of startle in rats with hippocampal damage has been reported (Leaton 1981) a comparison was made of the first and last 3 trials of the first 10 trial block during each session. In the first session there was a decline of 22% in startle amplitude of sham-irradiated rats [Mann-Whitney U $(21,26) = 349.5$, $P < 0.05$] and a decline of 8% for irradiated rats [not significant]. In the second session (massed trials) there was a 52% decline in startle amplitude for sham-irradiated rats [Mann-Whitney U $(9,12) = 96$, $P < 0.01$] and a 25% drop for irradiated rats [not a significant drop].

Not all tone presentations resulted in startles (i.e. movements that met the criteria established by our apparatus settings). During the first session (spaced trials) the irradiated rats tended to startle more frequently than the control rats but this difference was not statistically significant. During the second session (massed trials) the higher frequency of startle was reliable [$t(18) = 1.71$, $P = 0.05$] in the hippocampally damaged rats.

Discussion

We report here a potentiation of the acoustic startle response in rats with radiation-induced hypoplasia of hippocampal dentate granule cells. This observation was made during 2 test sessions (separated by 4 days) in which tones were presented in either a spaced (1 per 30 s) or massed (1 per 15 s) format. Habituation of the acoustic startle response was not observed in animals with hippocampal damage.

The method of fractioned partial-brain x-irradiation used here has been shown to produce a selective reduction in the number of granule cells in the dentate gyrus (see present data and also: Hicks and D'Amato 1969; Bayer and Altman 1975; Bayer and Peters 1977). However, this damage in the neonatal hippocampus may also cause secondary anatomical changes. Zimmer and his colleagues (1985, 1986) have shown that the brain may compensate for this early radiation-induced damage to the hippocampal granule cells by stimulating dendritic growth. Their results demonstrate that a reduction of a specific neuronal population can induce: (1) a compensatory increase in the neuropil layers containing the dendrites of the remaining neurons, (2) a corresponding relative increase in their axonal projections, and (3) a shift and expansion of afferent projections to an adjacent neuronal population. Thus, although our hippocampal radiation produces damage specific to the granule cells, subsequent brain changes, in reaction to this initial damage, may produce more-pervasive changes in neuroanatomy. Although these data reflect changes in hippocampal afferents and intra-hippocampal neuroanatomy, the most dramatic and direct radiogenic damage observed in our experiment can be found within the granule cell layer of the dentate gyrus.

Some authors (Groves et al. 1974; Leaton 1981) have previously reported that lesions of the hippocampus do not consistently alter startle, while others (Coover and Levine 1972) have found increased acoustic startle after this surgery. The lesion/test interval may be a distinguishing feature between experiments that have found potentiated startle amplitudes and those studies not reporting these effects in rats with hippocampal damage. Enhanced startle has been found when there was a significant interval between the brain lesion and the startle test. In both the current experiment and that of Coover and Levine (1972) the lesion/test interval was long (approximately 194 and 70 days, respec-

tively). On the other hand, Leaton (1981) used a shorter interval (approximately 14 days) between lesioning and testing and reported little change in acoustic startle after hippocampectomy. These results parallel other data suggesting that acute startle changes following lesions of the inferior colliculus may be opposite those measured later (for review see Davies 1984). Still, a recent study reporting an early enhancement of acoustic startle 1 week after hippocampal damage has brought into question the universality of this lesion/test-latency effect (Tilson et al. 1987).

Our startle data also suggest that the habituation recorded in control animals during the full course of massed tone presentations was not evident in irradiated rats with hippocampal damage. In fact, experimental subjects exhibited a trend toward potentiation of their startle responding in the second half of our test session. On the surface our results appear to differ from those of Groves et al. (1974) and Leaton (1981) who have reported normal within-sessison startle habituation in rats with hippocampal damage. A fine analysis of the data helps reconcile these apparently divergent results. The habituation reported by other laboratories was evident within the first 10 stimulus presentations. Our startle sensitization required more than 20 trials to observe. Review of our data collected during the first 10 trials of each test session also revealed a tendency for sham-irradiated animals to habituate to the acoustic stimulus. While not statistically significant, a similar trend was observed during the first 10 startle trials in the irradiated subjects as well. However, on subsequent trials, startle amplitudes increased in the animals with hippocampal damage while startles of control rats continued to gradually decline in magnitude. Independent of these within-session comparisons, we also analyzed between-session changes in startle. Although a trend toward a decline was observed in both experimental and control subjects, there was no statistically significant change in startle amplitude between our test sessions. These data are consistent with others that have not detected recovery of habituated startle responding between test sessions (Leaton 1981). It should be noted that rats with radiation-induced hippocampal damage readily exhibit habituation of spontaneous locomotion within the same time parameters that we observed little startle habituation (Mickley et al. 1989). However, others have commented that habituation of exploratory behavior and habituation of elicited reflex-like responses depend on different underlying mechanisms and that hippocampal lesions do not produce general habituation deficits (for review, see Leaton 1981).

The primary distinguishing features between the present startle study and others are the timing, method and anatomical result of the hippocampal lesion procedure. Our radiation-induced hippocampal lesion was produced in neonates and primary damage was confined to the granule cells of the dentate gyrus. Others (Leaton 1981; Groves et al. 1974; Coover and Levine 1972), relying on aspiration and electrolytic lesion techniques, have removed most of the hippocampus as well as portions of the cerebral cortex of the adult animal. Using a third procedure, additional investigators have used the neurotoxin colchicine to produce fairly selective damage to the granule cells of the adult hippocampal dentate gyrus. With neuroanatomical changes similar to the radiation-induced damage reported here, colchicine-injected rats have exhibited a significant enhancement of acoustic startle reactivity in one study (Tilson et al. 1987), but not in another that used auditory stimulus parameters different from our own (Walsh et al. 1986). Future experiments that systematically manipulate startle parameters as well as the timing and extent of hippocampal lesions may further develop our understanding of the role of hippocampus in startle responding. According to our data, however, the granule cells of the hippocampus exert an inhibitory influence on the primary acoustic startle circuit of the brainstem. With the loss of this tonic inhibitory influence, startle responding is greater in amplitude, more frequent and less likely to habituate.

Acknowledgements. The authors recognize the helpful technical assistance provided by Ms. Maureen A. Mulvihill and Mr. Thomas J. Nemeth. The dosimetry and irradiations were performed by Mr. Douglas Eagleson and Mr. Ernest Golightly. Statistical advice was provided by Mr. William Jackson. We thank Ms. Lilly Heman-Ackah for her excellent histological assistance. This research was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under work unit B4163. Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred.

References

Altman J (1986) An animal model of minimal brain dysfunction. In: Lewis M (ed) Learning disabilities and prenatal risk. University of Illinois Press, Urbana Chicago, pp 241–304

Altman J, Anderson WJ, Wright KA (1968) Gross morphological consequences of irradiation of the cerebellum in infant rats with repeated doses of low-level x-ray. Exp Neurol 21: 69–91

Altman J, Brunner RL, Bayer SA (1973) The hippocampus and behavioral maturation. Behav Biol 8: 557–596

Bayer SA, Altman J (1975) Radiation-induced interference with postnatal hippocampal cytogenesis in rats and its long-term effects on the acquisition of neurons and glia. J Comp Neurol 163: 1–20

Bayer SA, Peters PJ (1977) A method for x-irradiating selected brain regions in infant rats. Brain Res Bull 2: 153–156

Bayer SA, Brunner RL, Hine R, Altman J (1973) Behavioural effects of interference with the postnatal acquisition of hippocampal granule cells. Nature New Biol 242: 222–224

Blanchard RJ, Fial RA (1968) Effects of limbic lesions on passive avoidance and reactivity to shock. J Comp Physiol Psychol 66: 606–612

Brunner RL, Altman J (1974) The effects of interference with the maturation of the cerebellum and hippocampus on the development of adult behavior. In: Stein DG, Rosen JJ, Butters N (eds) Plasticity and recovery of function in the central nervous system. Academic Press, New York, pp 129–148

Casarett GW (1980) Radiation histopathology, Vol II, CRC Press. Boca Raton

Coover GD, Levine S (1972) Auditory startle response of hippocampectomized rats. Physiol Behav 9: 75–77

Davis M (1984) The mammalian startle response. In: Eaton RC (ed) Neural mechanisms of startle behavior. Plenum Press, New York London, pp 287–351

Douglas RJ (1967) The hippocampus and behavior. Psychol Bull 67: 416–442

Eaton RC (ed) (1984) Neural mechanisms of startle behavior. Plenum Press, New York London

Groves PM, Wilson CJ, Boyle RD (1974) Brain stem pathways, cortical modulation, and habituation of the acoustic startle response. Behav Biol 10: 391–418

Handelmann GE, Olton DS (1981) Spatial memory following damage to hippocampal CA3 pyramidal cells with kainic acid: impairment and recovery with preoperative training. Brain Res 217: 41–58

Hicks SP (1958) Radiation as an experimental tool in mammalian developmental neurology. Physiol Rev 38: 337–356

Hicks SP, D'Amato CJ (1966) Effects of ionizing radiations on mammalian development. In: Woollam DHM (ed) Advances in teratology. Logos Press, London, pp 195–250

Isaacson RL (1974) The limbic system. Plenum Press, New York

Isaacson RL, Wickelgren WO (1962) Hippocampal ablation and passive avoidance. Science 138: 1104–1106

Isaacson RL, Douglas RJ, Moore RY (1961) The effect of radical hippocampal ablation on acquisition of avoidance responses. J Comp Physiol Psychol 54: 625–628

Kimble DP (1968) Hippocampus and internal inhibition. Psychol Bull 70: 285–295

LaBoissière E (1976) Histological processing for the neural sciences. Thomas, Springfield, Ill.

Landis C, Hunt WA (1939) The startle pattern. Farrar and Rinehart, New York

Leaton RN (1981) Habituation of startle response, lick suppression, and exploratory behavior in rats with hippocampal lesions. J Comp Physiol Psychol 95: 813–826

Means LW, Leander JD, Isaacson RL (1971) The effects of hippocampectomy on alternation behavior and response to novelty. Physiol Behav 6: 17–22

Mickley GA, Ferguson JL, Nemeth TJ, Mulvihill MA, Alderks CE (1989) Spontaneous perseverative turning and other behavioral changes in rats with radiation-induced hippocampal damage. Behav Neurosci (in press)

Mosier HD, Jansons RA (1970) Effect of x-irradiation of selected areas of the head of the newborn rat on growth. Radiat Res 43: 92–104

O'Keefe J, Nadel L (1978) The hippocampus as a cognitive map. Oxford University Press, Oxford

Paxinos G, Watson C (1982) The rat brain in stereotaxic coordinates. Academic Press, Sidney

Teitelbaum H, Milner P (1963) Activity changes following partial hippocampal lesions in rats. J Comp Physiol Psychol 56: 284–289

Tilson HA, Rogers BC, Grimes L, Harry GJ, Peterson NJ, Hong JS, Dyer RS (1987) Time-dependent neurobiological effects of colchicine administered directly into the hippocampus of rats.Brain Res 408: 163–172

Wallace RB, Altman J (1970a) Behavioral effects of neonatal irradiation of the cerebellum. I. Qualitative observations in infant and adolescent rats. Dev Psychobiol 2: 257–265

Wallace RB, Altman J (1970b) Behavioral effects of neonatal irradiation of the cerebellum. II. Quantitative studies in young-adult and adult rats. Dev Psychobiol 2: 266–272

Walsh TJ, Schuiz DW, Tilson HA, Schmechel DE (1986) Colchicine-induced granule cell loss in rat hippocampus: selective behavioral and histological alterations. Brain Res 398: 23–36

Weisbroth SH (1979) Bacterial and mycotic diseases. In: Baker HJ, Lindsey JR, Weisbroth SH (eds) The laboratory rat: biology and diseases, Vol I. Academic Press, New York, pp 206–208

Wilkins DE, Hallett M, Wess MM (1986) Audiogenic startle reflex of man and its relationship to startle syndromes. Brain 109: 561–573

Zimmer J, Sunde N, Sorensen T (1985) Reorganization and restoration of central nervous connections after injury: a lesion and transplant study of the rat hippocampus. In: Will BE, Schmitt P, Dairymple-Alford JC (eds) Brain plasticity, learning and memory. Plenum Publishing, London, pp 505–518

Zimmer J, Laurberg S, Sunde N (1986) Non-cholinergic afferents determine the distribution of the cholinergic septohippocampal projection: a study of the AChE staining pattern in the rat fascia dentata and hippocampus after lesions, X-irradiation, and intracerebral grafting. Exp Brain Res 64: 158–168

Received April 13, 1988 / Accepted August 22, 1988